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# Endemic, Epidemic Clone of *Salmonella enterica* Serovar Typhi Harboring a Single Multidrug-Resistant Plasmid in Vietnam between 1995 and 2002

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***Salmonella enterica*** **serovar Typhi strains resistant to ampicillin, chloramphenicol, tetracyclines, streptomycin, and cotrimoxazole, isolated from sporadic cases and minor outbreaks in Vietnam between 1995 and 2002, were typed and compared. Plasmid fingerprinting, Vi bacteriophage typing, XbaI pulsed-field gel electrophoresis, and PstI ribotyping showed that endemic, epidemic multidrug-resistant typhoid fever was due, for at least 74.1% of the isolates, to one or two clones of serovar Typhi harboring a single resistance plasmid. PstI ribotyping was used as a basic technique to ensure that a serovar Typhi expansion was clonal.**

In many developing countries, extended local outbreaks of typhoid fever occur against a background of sporadic cases. For instance, in 1972 the endemic form turned epidemic in southern Vietnam (22). Moreover, resistance of *Salmonella enterica* serovar Typhi to chloramphenicol appeared in this country at the end of 1971. During the following years, resistant strains have been increasingly isolated (211 resistant strains of 288 isolates between 1972 and 1975, i.e., 73.3%, reaching 85.4% in 1975). These strains were found to be multidrug resistant (MDR), i.e., resistant to chloramphenicol, tetracyclines, streptomycin, and sulfonamides. Resistance was encoded by self-transferable plasmids (22, 39).

Several studies have been published about typhoid fever and MDR strains worldwide, but none from Vietnam was published until the beginning of the 1990s; during this time, serovar Typhi isolates became susceptible again in Vietnam, with a few MDR strains (Le Thi Anh Hong, personal communication). In 1993, an epidemic of typhoid fever in the Kien Giang province (southern Vietnam) was again due to MDR isolates, i.e., resistant to ampicillin, chloramphenicol, tetracyclines, and trimethoprim-sulfamethoxazole (cotrimoxazole) (28, 34). MDR strains have been increasing, accounting for over 80% of all isolates by 1994 in southern Vietnam (49). At this time, less than 5 and 10% of MDR strains were isolated in the center and the north of Vietnam, respectively (30, 35). In and after 1995, over 90% of the isolates from both regions were MDR with the previously observed resistance pattern; in particular, an outbreak in 1996 in Hue Province consisted of MDR strains (29). Finally, since 1996, strains resistant to quinolones were more and more frequently isolated in Vietnam, and most of them were MDR (51).

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It is useful to understand the mode of spread of serovar Typhi strains in order to implement rational strategies for the prevention of typhoid fever. Is it caused by multiple serovar Typhi clones or a single one? For this purpose, several methods have been used. For instance, in southern Vietnam, Wain et al. (50), using pulsed-field gel electrophoresis (PFGE), bacteriophage typing, and ribotyping, described a variety of different serovar Typhi clones in sporadic typhoid cases.

The aim of this study was to determine whether the endemic, epidemic MDR typhoid fever found throughout Vietnam from 1995 to 2002 was due mainly to a single resistance plasmid and to a single serovar Typhi clone or not. The answer can be useful to indicate suitable measures in the field of public health. We picked epidemiologically independent isolates, performed restriction typing of plasmids, and compared serovar Typhi strains by phage typing and ribotyping.

## MATERIALS AND METHODS

**Bacterial strains, susceptibility tests, and flow chart of strains and methods.** Between 1995 and 2002, 519 *S. enterica* serovar Typhi clinical isolates were collected throughout Vietnam in the Laboratoire d'Épidémiologie de la Résistance Bactérienne (ERB-lab), Institut National d' Hygiène et d'Épidémiologie (INHE), Hanoï, Vietnam, i.e., 363 isolates during the period from 1995 to 1997 and 156 from 1998 to 2002. Four hundred twenty-six of the strains collected were MDR: 194 from the north, 116 from the center, and 116 from the south; 78.7% were MDR during the period from 1995 to 1997, and 89.9% were MDR during 1998 to 2002. One hundred forty-two MDR and 18 susceptible serovar Typhi strains, epidemiologically independent, were selected and studied. They were identified with the API 20E system (BioMerieux, Marcy l'Etoile, France).

Agglutination was performed with antisera specific for the O:9, Vi, and H:d antigens (Bio-Rad, Hanoï, Vietnam). Susceptibility tests were carried out by the disk (Bio-Rad) diffusion method and interpreted according to published standards and the comparative interpretative reading method (42, 43). The latter is based on multivariate data analyses which separate the different bacterial phenotypes of susceptibility and resistance, generated by genetic determinants and biochemical mechanisms; this approach is especially relevant for work on the epidemiology of antimicrobial resistance, which is our purpose.

Antibiotics implicated in the resistance of serovar Typhi isolates were tested, i.e., ampicillin, chloramphenicol, tetracycline, cotrimoxazole, nalidixic acid, and streptomycin (not used in therapy now).

TABLE 1. Ribotypes and phage types of 81 epidemiologically independent MDR *S. enterica* serovar Typhi isolates selected in Vietnam from 1995 to 2002

| Region | No. of isolates | Ribotype | No. of isolates | Phage type | No. of isolates | Plasmid MDR phenotype[a] | No. of isolates |
|---|---|---|---|---|---|---|---|
| North | 30 | 3a | 28 | E1 | 26 | Amp, Cm, Tc, Sm, Tmp-Su | 29 |
| | | | | E3 | 1 | Amp, Cm, Tmp-Su | 1 |
| | | Others | 2 | Others | 3 | | |
| Center | 18 | 3a | 18 | E1 | 1 | Amp, Cm, Tc, Sm, Tmp-Su | 12 |
| | | | | E3 | 9 | Amp, Cm, Tc, Sm | 1 |
| | | Other | 0 | Others | 8 | Cm, Tc, Sm, Tmp-Su | 4 |
| | | | | | | Tc, Sm | 1 |
| South | 33 | 3a | 33 | E1 | 11 | Amp, Cm, Tc, Sm, Tmp-Su | 30 |
| | | | | E3 | 14 | Amp, Tc, Sm, Tmp-Su | 1 |
| | | Other | 0 | Others | 8 | Cm, Tc, Sm, Tmp-Su | 2 |

[a] Resistant to ampicillin and ticarcillin (Amp), chloramphenicol (Cm), tetracyclines (Tc), streptomycin (Sm), and trimethoprim-sulfamethoxazole (Tmp-Su).

All 142 MDR serovar Typhi isolates selected were conjugated to *Escherichia coli*; plasmids were extracted from 107 of these 142 strains and from their *E. coli* transconjugants; plasmids from 54 of these 107 *E. coli* transconjugants were fingerprinted. Eighty-one of the 142 selected MDR isolates and all 18 of the selected susceptible serovar Typhi isolates were phage typed and ribotyped. Eleven of these 81 MDR strains were tested by PFGE. Three MDR strains isolated from 1974 to 1975 in Saïgon (now Ho Chi Minh City), Vietnam, were added to the study.

The reference strains were *E. coli* R39 harboring four plasmids of different sizes, 98, 46, 24, and 4.6 MDa, kindly supplied by the Laboratory of Bacteriology, Center for Tropical Diseases, Ho Chi Minh City, Vietnam; *E. coli* K-12 J5-3, lactose positive, resistant to rifampin, kindly supplied by the Laboratoire de Bacteriologie, Hôpital Universitaire Avicenne, Bobigny, France; and *E. coli* W3110, lactose positive, resistant to nalidixic acid, kindly supplied by the Department of Bacteriology, University of Medicine of Hanoi, Hanoi, Vietnam.

**Conjugational transfer of drug resistance.** Conjugational transfers were carried out in ERB-lab, INHE, Hanoï, from 142 selected MDR serovar Typhi isolates to plasmid-free strains of both rifampin-resistant and nalidixic acid-resistant *E. coli*. All mating procedures were performed at 37°C for 18 h before plating onto Drigalski medium (Bio-Rad) containing 20 mg of ampicillin, chloramphenicol, or tetracycline (Europ Continents, Hanoï, Vietnam) per liter. Rifampin or nalidixic acid (Europ Continents) was used for counterselection at a final concentration of 60 and 20 mg/liter, respectively. Frequency of transfer was expressed as the number of resistant transconjugants per number of donor cells. Antibiotic susceptibility tests were performed and interpreted for transconjugants as indicated for serovar Typhi strains.

**Extraction and determination of plasmid size.** Plasmids were extracted from 107 of 142 selected MDR serovar Typhi isolates and from their *E. coli* transconjugants, by the method of Kado and Liu (18) in ERB-lab, INHE, Hanoï. To separate individual plasmids in strains harboring more than one plasmid and estimate their sizes, the first step was to compare them to the four plasmids of known size harbored by *E. coli* R39 on 0.7% (wt/vol) agarose gels (Europ Continents) electrophoretically (80 V, 3 h). Then, restriction endonucleases KpnI, ScaI, and BglII (Roche Diagnostics, Meylan, France) were used to cleave the plasmids and compare the sizes of the fragments to the size markers, a 1-kb DNA ladder (New England Biolabs, Beverly, Mass.) containing 13 fragments (from 12.2 to 1.0 kbp); Raoul (QBiogène, Illkirch, France), containing 22 fragments (from 48.5 to 0.2 kbp); and Marker XV (Roche Diagnostics), containing 22 fragments (from 48.5 to 7.6 kbp).

**Plasmid fingerprinting.** Among the plasmids extracted from the previous 107 *E. coli* transconjugants, 54 plasmids (18 from the north, 14 from the center, and 22 from the south of Vietnam) of similar size were digested by EcoRI or HindIII endonuclease in ERB-lab, INHE, Hanoï, according to the manufacturer's instructions (New England Biolabs). The restriction digests were electrophoresed (40 V, 16 h) on 0.8% (wt/vol) agarose gels (Europ Continents) in order to estimate their size (41). HindIII-digested bacteriophage lambda DNA (New England Biolabs) was used as a size marker.

**Bacteriophage typing.** Eighty-one of 142 MDR and 18 selected susceptible serovar Typhi isolates were analyzed by Vi phage typing, with the method described by Craigie and Yen (8, 9), in the Unité de Biodiversité des Bactéries Pathogènes Émergentes (BBPE-Unit), Institut Pasteur, Paris.

**Ribotyping.** The same 81 MDR and 18 susceptible isolates as used in phage typing were used in ribotyping. All the serovar Typhi strains investigated were manually ribotyped, and 16 strains of different ribotypes were subjected to the RiboPrinter microbial characterization system (Qualicon Inc., Wilmington, Del.). Ribotype numbering and identification were generated by this system. Pattern images (manual ribotyping or automated ribotyping) were analyzed with the programs of the Taxotron package (14), and the patterns were compared to the database of the BBPE-Unit (16).

Manual ribotyping was performed as described previously (15, 37, 38) with slight modifications. DNA was cleaved with restriction endonuclease PstI (Amersham Biosciences, Orsay, France); electrophoresis of restricted DNA samples was carried out with 0.7% (wt/vol) agarose gels (Seakem ME agarose; FMC BioProducts, Rockland, Maine) in 0.5× Tris-borate-EDTA buffer for 16 h at 1.5 V/cm, and the digoxigenin-labeled-oligonucleotide probe was from MWG-Biotech (Ebersberg, Germany). Immunoenzymatic detection was performed with a detection kit (Roche Diagnostics) according to the supplier's instructions. MluI-digested genomic DNA of *Citrobacter koseri* CIP105177 (Institut Pasteur, Paris) was used as a size marker, with fragments of 16.8, 12.5, 7.3, 6.6, 5.8, 5.1, 4.4, 3.0, 2.8, 1.7, 1.4, and 1.2 kbp, to determine the sizes of the DNA fragments.

Automated ribotyping was performed with the RiboPrinter according to the manufacturer's instructions. Digestion with restriction endonuclease PstI, gel separation, transfer, and hybridization with a chemiluminescently labeled DNA probe containing the *E. coli* rRNA operon were carried out in 8 h.

**PFGE fingerprinting.** The PFGE technique of contour-clamped homogenous electric fields (CHEF) was used at the BBPE-Unit for the fingerprinting of 11 isolates, three from the north, four from the center, and four from the south, among the 81 ribotyped MDR isolates. XbaI endonuclease (Amersham Pharmacia Biotech, Uppsala, Sweden) was used for digestion of genomic DNA as described by Nair et al. (25), and the DNA fragments were resolved with a CHEF-DRIII apparatus (Bio-Rad Laboratories, Richmond, Calif.) at 6 V/cm for 24 h at 14°C with a pulse time of 20 to 65 s and an electric field angle of 120°. Multimers of phage lambda and Low Range (New Englands Biolabs, Inc., Beverly, Mass), and *Salmonella* serovar Branderup digested by XbaI were used as molecular size standards.

## RESULTS

**Resistance phenotypes and transfer of resistance plasmids.** All the resistance-related antibiotics had MICs of between 128 and >512 mg/liter for the MDR serovar Typhi isolates and their *E. coli* transconjugants.

Most MDR isolates (129 of 142) exhibited the same resistance pattern; they were resistant to ampicillin and ticarcillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole (cotrimoxazole). The other patterns (13 of 142 isolates) differed by one or several resistance markers (Table 1). More and more isolates have expressed resistance to nalidixic acid and fluoroquinolones since 1996.

All but five of the MDR isolates transferred en bloc the multidrug resistance (mainly ampicillin, chloramphenicol, tetracyclines, streptomycin, and trimethoprim-sulfamethoxazole) to rifampin- or nalidixic acid-resistant *E. coli*. Resistance to nalidixic acid and fluoroquinolones was never transferred to

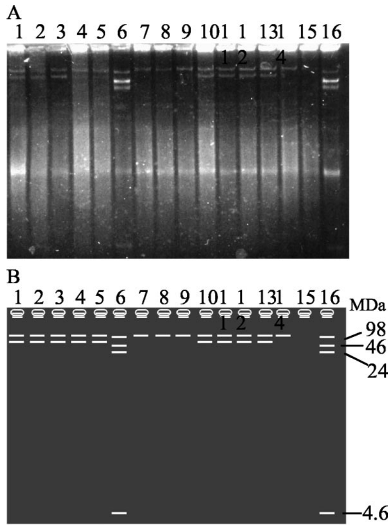


FIG. 1. Electrophoresis on an agarose gel (0.7%, 80 V, 3 h) of plasmid DNA extracted from MDR serovar Typhi isolates and *E. coli* transconjugants. Lanes 6 and 16, four plasmids (98, 46, 24, and 4.6 MDa) from reference strain *E. coli* R39; lane 15, no plasmid from reference strain *E. coli* K12 J5-3; lanes 1, 3, 11, and 13, both 122- and 63-MDa plasmids from isolates; and lanes 2, 4, 5, and 12, both 122- and 63-MDa plasmids from their transconjugants; lanes 7 and 8, only the 122-MDa plasmid from an isolate and its transconjugant, respectively; lane 10, both plasmids from an isolate; and lane 9, only the 122-MDa plasmid from its transconjugant; lane 14, the single 122-MDa plasmid from an isolate. (A) Photograph of the agarose gel. (B) Image analyzed by computer.

rifampin-resistant *E. coli*. The frequency of transfer ranged from $10^{-8}$ to $10^{-5}$ drug-resistant conjugants per donor cell. All but 15 of the *E. coli* transconjugants expressed the same resistance pattern as that of the serovar Typhi donors. The other 15 transconjugants expressed resistance patterns which differed by one or several resistance markers.

The main resistance pattern differed from the phenotype exhibited in 1974 to 1975 (which included resistance to chloramphenicol, tetracycline, streptomycin, and sulfonamide) by acquiring new resistance characters probably related to new drugs without losing the previous ones even though these resistance-related drugs are no longer used.

**Plasmid profile analysis.** The 107 MDR serovar Typhi isolates investigated and their transconjugants harbored a large plasmid (184.8 ± 8.2 kbp, i.e., ≈122 MDa), and 50 of these serovar Typhi isolates contained a second plasmid (94.6 ± 3.5 kbp, i.e., ≈63 MDa); this second plasmid was not always transferred to *E. coli* (Fig. 1). The transconjugants which harbored the single 122-MDa plasmid were MDR.

Some other serovar Typhi isolates which harbored the single 63-MDa plasmid were susceptible to antibiotics. Incidentally, a few serovar Typhi isolates were recognized as MDR at the moment of isolation, but after conservation, they were found not to carry any 122-MDa plasmid; we retested these strains and recognized them as being susceptible to antibiotics. Therefore, multidrug resistance was associated with a 122-MDa self-transferable plasmid.

**Plasmid fingerprints.** HindIII restriction digests of the 122-MDa plasmids extracted from the *E. coli* transconjugants are shown in Fig. 2. The EcoRI restriction digests are not shown. All these plasmids were identical with respect to both restriction digests.

**Vi phage types.** Vi phages types E1 and E3 were predominant among the MDR serovar Typhi isolates (62 of 81 strains, i.e., 76.5%) (Table 1). Since 1995, most isolates from the north exhibited phage type E1 and only one showed the E3 type (1999); isolates that originated from the center had mainly phage type E3 and one had the E1 type (1996); isolates from the south were a mixture of both phage types from 1995 to 2002. Nine of the 81 isolates studied (11.1%) were phage untypeable ($Vi^-$), and four isolates that were Vi positive were not sensitive to the phages used. Other types definitely different from the E types were observed for six isolates between 1995 and 2002, i.e., phage types M4, D4, and 46.

Eighteen serovar Typhi isolates susceptible to antibiotics and harboring no plasmid or only a 63-MDa plasmid exhibited various phage types, i.e., A, M2, M3, and also E1 or E3, or were not sensitive to the phages used.

The three strains isolated in 1974 to 1975 in Saïgon were $Vi^-$ or had the phage type 38, not found in the present study.

**Ribotypes.** Ribotype 3a (five bands) was associated with 79 of 81 (97.5%) MDR isolates (Table 1, Fig. 3). Two other ribotypes isolated in 1995 in the north were 230 and 236, associated with phage type E1. Therefore, ribotype 3a associated with phage types E1 and E3 was observed for 60 of 81 MDR serovar Typhi isolates, i.e., 74.1%. All nine phage-untypeable ($Vi^-$) isolates exhibited ribotype 3a.

Among the 18 isolates susceptible to antibiotics, 10 exhibited various ribotypes, 3ab, 26a, 187, 228, 237, 240, and 241; eight were 3a, seven of these exhibiting phage type E1 or E3, which suggests loss of the large resistance plasmid.

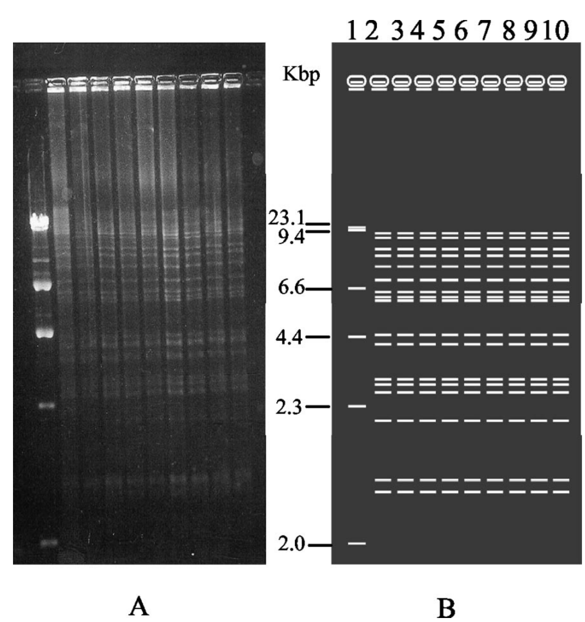


FIG. 2. Electrophoresis on an agarose gel (0.8%, 40 V, 16 h) of HindIII restriction digests of 122-MDa plasmid DNA extracted from *E. coli* transconjugants of MDR serovar Typhi isolates. Lane 1, HindIII-digested bacteriophage lambda DNA. (A) Photograph of the agarose gel. (B) Image analyzed by computer.

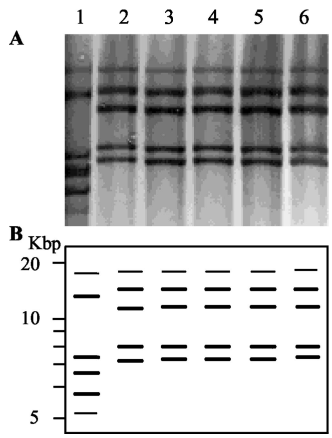


FIG. 3. PstI ribotype 3a of five isolates (lanes 2 to 6) obtained by the manual method with electrophoresis on an agarose gel (0.7%, 1.5 V/cm, 16 h) (A) and the corresponding schematic representation established by Taxotron software (B). Lane 1, molecular size markers.

The three MDR strains isolated in 1974 to 1975 in Saïgon had various ribotypes, 151, 239, and 263.

**PFGE fingerprints.** The number of DNA fragments obtained by XbaI digestion ranged between 11 and 13 (results not shown). Among the 11 isolates of ribotype 3a which were PFGE typed, four patterns were observed. PFGE pattern 1 was shown by three phage type E1 isolates from north and south Vietnam and five phage type E3 isolates from central and south Vietnam. PFGE pattern 2 was shown by one phage type E1 isolate, PFGE pattern 3 was shown by one phage type E1 isolate, and PFGE pattern 4 was shown by one phage type E3 isolate. PFGE patterns 2 to 4 differed from PFGE pattern 1 by only two or three fragments.

## DISCUSSION

After the MDR typhoid fever epidemic occurred in Mexico during 1972 (31), large autotransferable resistance plasmids harbored by MDR serovar Typhi strains have been isolated worldwide, mainly in developing countries where typhoid fever remains endemic: South America (13), Africa (1), the Middle East (33), southern Asia (2, 17, 19, 23, 40, 48), and southeast Asia (20, 21, 36). Most of these plasmids but not all belonged to the H1 incompatibility group, but their molecular size varied between 73 and 192 MDa; the size could differ among plasmids obtained from the same epidemic and from one country to another but was stable during every outbreak; in particular, an estimated 140-MDa plasmid was harbored by nearly all the MDR isolates from southern Vietnam between 1993 and 1997 (6). Therefore, the incompatibility grouping of plasmids is a useful epidemiological tool (5, 7, 10) but is less precise than restriction pattern analysis. The restriction patterns obtained for the 122-MDa plasmids were identical for all the strains studied. We used two different endonucleases, not a single one, to confirm plasmid identity. We can conclude that the MDR serovar Typhi strains isolated throughout Vietnam between 1995 and 2002 harbored a single resistance plasmid. Has a strain which harbored this plasmid taken advantage of this fact to achieve a clonal expansion?

The human-adapted serovar Typhi was considered by Selander et al. (44) to be one of the least genotypically heterogeneous *Salmonella* serovars; however, they pointed out considerable variation in its rRNA operon. Boyd et al. (4) investigated the genomic content of a set of isolates: they found such differences that they concluded the genomic reservoir was unstable, even within a highly clonal bacterial population. Several workers using ribotyping with or without PFGE (3, 11, 12, 16, 26, 27, 32) demonstrated that PstI ribotyping is a reliable technique, i.e., stable, reproducible, and sensitive for subtyping serovar Typhi. PstI ribotyping displays a high discriminatory power even if it can only detect changes at restriction sites at different copies of the rRNA gene, while differences in restriction sites at regions other than the ribosomal operon could be detected by digestion of chromosomal DNA (21).

A good correlation was established between the heterogeneous patterns obtained from both XbaI PFGE and PstI ribotyping of isolates involved in sporadic cases (46). The results of Ling et al. (21) (95 and 124 patterns obtained by PstI ribotyping and NaI PFGE, respectively, from 290 isolates), those of Mirza et al. (24) (five clones divided into 11 subgroups from 193 MDR isolates with XbaI PFGE) and those from Thong et al. (47) (11 of 23 paired isolates from individual patients exhibited genetic changes with XbaI PFGE) lead us to estimate that the higher discriminatory power of PFGE displays clonal variations more than independent clones, whereas the PstI ribotyping is a solid tool to separate real clones; thus, Pang et al. (32) separated eight clones from 20 isolates by PstI ribotyping. Moreover, ribotyping could not only recognize homogeneous clones related to outbreaks, but also discriminate isolates from sporadic cases (26, 27).

The results of the present study are in accord with these works. Eight isolates were indistinguishable by PFGE. For three isolates, two or three band variations in PFGE patterns are not epidemiologically significant. According to Tenover et al. (45), all isolates of ribotypes 3a that were PFGE typed were closely related irrespective of their classification as phage type E1 or E3.

From 1997 to the end of 2003, 1,154 serovar Typhi strains had been collected, phage typed, and ribotyped and were differentiated into 302 ribotypes in the BBPE-Unit (16). In this serovar Typhi strain collection, Vi phage types E1 and E3 represent 16 and 0.7%, respectively; ribotype 3a represents 11.3%; and the associated phage type E1-ribotype 3a represents 5.5%. These figures indicate that ribotyping in combination with phage typing had high discriminatory power and so is an accurate epidemiological tool, in any case for *S. enterica* serovar Typhi. The use of both methods appears suitable to determine whether serovar Typhi isolates belong to the same clone or not.

Our results have shown that the association of ribotype 3a with phage type E1 or E3 was strongly predominant (74.1%) among MDR serovar Typhi isolates collected throughout Vietnam from 1995 to 2002, while the phage types of MDR serovar Typhi strains isolated in 1974 to 1975 in Saïgon were different. We can assume that most of the MDR typhoid fever cases which occurred over 8 years in Vietnam were due to the clonal expansion of one or two strains, depending on whether the distinction between Vi phage types E1 and E3 is reliable or not. If the distinction is reliable, two predominant clones have coexisted in the south, at least in Ho Chi Minh City and in three provinces, since 1995 and even before (6), phage type E1 strongly predominating in the north and phage type E3 in the center.

If the distinction between phage types E1 and E3 is not reliable, and taking into account a proportional part of the Vi-untypeable strains, all harboring the 3a ribotype, most MDR serovar Typhi isolates (four-fifths) exhibited genetic homogeneity. This means that the endemic, epidemic MDR typhoid fever in Vietnam was due for the most part to a single clone spreading from the south to the center and the north from 1995 to 2002. This is supported by results from the BBPE-Unit collection (Institut Pasteur, Paris): among ribotype 3a strains, phage types E1 and E3 represent 49.2 and 0%, respectively; of 29 3a/E1 serovar Typhi isolates with travel history, 20 were associated with travel in Asia (India, Pakistan, and Laos). Moreover, serovar Typhi type E3 is less susceptible to Vi bacteriophages than type E1. Thus, clone 3a-E1, found in Vietnam as well as in Asian countries, could have evolved in Vietnam into a new clone, 3a-E3, which was never seen in our collection before. Therefore, one clonal expansion (one ribotype) which evolved from one phage type to another and underwent limited chromosomal rearrangements (pointed out by PFGE fingerprints) is a valid hypothesis.

In countries where typhoid fever is endemic, isolates from the same outbreak appeared to be genetically identical, whereas sporadic cases were caused by a wide variety of types (6, 11, 12, 17, 21, 27, 32, 46). We detected various phage types and ribotypes among the serovar Typhi isolates from sporadic cases, but only among the antibiotic-susceptible ones. In contrast, the results from plasmid fingerprinting, phage typing, and PstI ribotyping show that most of the MDR serovar Typhi strains isolated from both sporadic cases and minor outbreaks over 8 years throughout Vietnam, and of course from the epidemic which occurred in Hue in 1996, belonged to one or at the most two clones and harbored a single resistance plasmid.

The outcome of our study will lead to the implementation of rational strategies and suitable measures in the field of public health in order to prevent MDR typhoid fever, insofar as clonal spreading of MDR *S. enterica* serovar Typhi is likely to be due to a network of carriers rather than to multiple sources of infection.

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